12 THIN LAYER CHROMATOGRAPHY

12.1 Objective

12.1.1 To familiarize the trainee with the theory and application of thin layer chromatography in drug analysis

12.2 Modes of Instruction

12.2.1 Self-directed study through reading assignments and worksheets

12.2.2 Presentations and demonstrations

12.2.3 Practical exercise

12.3 Reference

12.3.1 *Basic Training Program for Forensic Chemists*, U.S. Department of Justice, Drug Enforcement Adminstration, Office of Science and Technology, pp. 4-39 through 4-49.

12.3.2 Moffat, A. C., editor. *Clarke's Isolation and Identification of Drugs*. London: The Pharmaceutical Press, 1986, pp. 160-177.

12.3.3 DFS Controlled Substances Procedures Manual, Thin Layer Chromatography Section.

12.3.4 Randerath, Kurt. *Thin-Layer Chromatography, Second Edition*. New York: Academic Press, 1968.

12.3.5 *Methods of Analysis for Alkaloids, Opiates, Marihuana, Barbiturates, and Miscellaneous Drugs.* Internal Revenue Service, (Reprinted by the Bureau of Narcotics and Dangerous Drugs, U. S. Department of Justice), rev. 6-67.

12.3.6 Stahl, Egon, *Thin-Layer Chromatography*, 2nd ed., Berlin: Springer-Verlag, 1969.

12.3.7 Bauer, Karin, *et. al*. *Thin Layer Chromatography*, Heidelberg, Germany: EM Science, , 1991.

12.4 Assignments

12.4.1 Completion of required reading assignments (12.3.2, 12.3.3)

12.4.2 Study questions

12.4.3 Completion of Known Notebook

12.5 Study Questions

12.5.1 Define the following:

- Chromatography
- Thin Layer Chromatography
- Stationary phase
- Mobile phase
- Solvent front
- R_f value
- Adsorption
- Absorption

- Elution
- Partition coefficient (K)
- Polarity
- Dipole moment
- Dielectric constant
- Visualizing reagent

12.5.2 For the silica gel GF TLC plates, what are the "GF" components and what is their purpose?

12.5.3 What is meant by "quenching fluorescence"? Which drugs do not quench UV fluorescence?

12.5.4 Why is silica used preferentially over alumina in drug analysis?

12.5.5 What is the general limit of detection of TLC? What factors influence this?

12.5.6 What are "tailing" and "bearding"? What causes these to occur and what can be done to minimize it?

12.5.7 Where can the recipes for each of the following TLC baths found?

12.5.7.1 TLC1

12.5.7.2 TLC2

12.5.7.3 TLC3

12.5.8 What is an elutropic series? How will the polarity of solvents change when they are mixed together?

12.5.9 Which drugs fluoresce under long wave UV? What is the difference in wavelength between short and long wave UV?

12.5.10 Where can the recipes for each of the following TLC visualizing reagents found? For what type(s) of drugs are each utilized most effectively?

- Iodoplatinate
- Dragendorff
- Potassium permanganate
- Ehrlich's
- Fluram
- Ceric sulfate
- Iodine vapors
- Furfural
- Mercuric Sulfate / Diphenylcarbazone
- Ninhydrin

12.5.11 Explain the interaction of the sample, mobile phase, and stationary phase in TLC.

12.5.12 What chemical properties of a drug affects its migration? Explain any and all intermolecular forces which may be at work.

12.5.13 How can LSD and LAMPA be separated?

12.5.14 Why do spots having a larger R_f value generally have larger diameters than spots with relatively low R_f values?

12.5.15 Does sample concentration have an effect on TLC migration? If so, why?

12.5.16 What factors influence an Rf value's reproducibility? How can these factors be controlled?

12.5.17 How can the results of TLC be documented?

12.5.18 What is two-dimensional TLC and why and how is it sometimes performed?

12.5.19 Explain as to a jury how TLC operates.

12.5.20 Where can the QA procedures for TLC baths and visualization reagents be found?

12.6 Practical Exercise

12.6.1 Prepare a TLC bath and visualizing reagent designated by the TC and perform all necessary QA and documentation prior to use.

12.6.2 Using the standards of the substances listed in Appendix 2, perform TLC analysis on each. Record your results in the Drug Known notebook. Do the tests by drug group so that differences in chemical structure can be correlated to different test results. Use the TLC1, TLC2, TLC3 baths and the following TLC sprays:

- $KMnO_4$
- Acidified Iodoplatinate
- Ceric Sulfate
- Ehrlich's
- Dragendorf
- Fluram / Long wave UV
- Furfural (carbamates only)
- Mercuric Sulfate / Diphenylcarbazone (barbiturates only)

12.6.3 Using the results from Section 12.6.1, answer the following questions:

- Explain the theory of using multiple TLC systems. Use examples from your data in your explanation.
- Discuss the separation effectiveness of TLC taking into account the structure of the molecule, the polarity/basicity of the solvent system and the polarity of the stationary phase. Use morphine, heroin and pentazocine as examples.
- The pairs Codeine/Ethylmorphine and Morphine/Codeine differ by only one carbon. Explain the differences between separating the two pairs.
- Which barbiturates can be visualized using $KMnO_4$? Why?
- Which bath(s) separate the phenethylamine-type compounds the best?

12.6.4 Obtain a mixture of cocaine/procaine from the TC. Perform 2-D TLC analysis to separate the components and confirm each by FTIR. Which bath is the best choice when a base determination is necessary? Why?

12.6.5 Obtain standards of GHB, GBL and 1,4-butanediol. Perform TLC using a mobile phase of ethyl acetate and visualize using iodine vapors.

12.6.6 Obtain standards of ephedrine and pseudoephedrine from the TC. Using the procedure outlined in the Procedures Manual, separate these compounds using TLC.

12.7 Modes of Evaluation

12.7.1 Written examination

12.7.2 Court exercise (mini-mock trial)

♦ End